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PHENOMENA AND DEVELOPMENT OF FECUNDATION.

By H. J. WEBBER.

(Continued from page 111.)

ORIGIN OF FECUNDATION.

Having now discussed shortly the nature of the sexes and the effect of environment on them, we are ready to inquire into the origin of fecundation. There are several well-marked stages which we may select, that appear to indicate the probable course of the development.

1. Among certain of the *Mycetozoa* or *Myxomycetes*, the Slime Molds, we find some very suggestive forms that are apparently near the beginning of the differentiation. They are even more interesting, if possible, coming as they do from a class of organisms placed in either kingdom as the lowest group, their animal or vegetable nature being in question, although authorities seem to incline toward believing them of slightly preponderating animal nature. In the lower Slime Molds belonging to the group *Acrasieæ*, the life history is shortly this: From the spore (fig. 13, *a*), on germination there creeps out a naked motile mass of protoplasm, which takes nourishment, grows and reproduces rapidly by dividing, the products of the division being in each case similar swarm spores (fig. 13, *b-f*). After an extended vegetation of this sort, a number of the swarm spores collect into a "herd" and creep about in company for a time, after which two of them, apparently through accident, come closer together and adhere. Now the others close in and unite with these two, forming what is termed a *plasmodium* (fig. 13, *g*). But in this union each swarm spore retains its individuality, the union being merely an adhesion, not even a fusion of the individual protoplasts. They creep around in this plasmodium form for a time until ready to complete the cycle by forming the mature stage, which is accomplished by the plasmodium coming to rest,

collecting into a conical mass and each original swarm spore forming a single encysted spore (fig. 13, *h*).

Why this mechanical adhesion of the swarm spores into a plasmodium? It would seem a scheme adopted by the plant to better protect the encysted spores.

2. In the *Myxomycetes* proper (the higher Slime Molds), the mode of life is practically the same as in the *Acrasieæ*, but here, when the swarm spores fuse to form the plasmodium; the fusion is complete so far as the protoplasts are concerned, but still there is a lack of a thorough fusion of all the elements as the nuclei remain apparently ununited (fig. 14).

In some *Myxomycetes* we find an indefinite number of swarm spores uniting to form the plasmodium, but in others the number thus fusing is reduced to a very few. Thus coupled with the growing complexity of the fusion or *pseudo-conjugation* of the swarm spores we have a reduction, also, in the number of elements fusing.

3. Between this process and that described as conjugation there are many interesting intermediate forms. Sometimes three or four spores of low Algæ unite as if to gather sufficient strength to make a combined start in life. In *Dictyosiphon hippuroides* Areschoug¹ has observed and figured the union of three zoospores. In *Acetabularia mediterranea* DeBary and Strasburger² have figured the copulation of several swarm spores (figs. 15 and 16). This multiple conjugation has also been observed in *Hydrodictyon*, *Spirogyra* and some other algæ, and while considered as abnormal, is apparently by no means uncommon. Among animals the young form of the sun animalcule (*Actinosphærium*) though usually uniting in twos, have been observed by Gabriel to sometimes exhibit multiple conjugation. In this stage the number uniting is reduced to a very few, usually not more than three or four, and is probably accompanied by nucle fusion.

4. In *Ulothrix* we find the differentiation carried still further. Here the protoplasm of certain cells of the parent plant divides up into numerous little pyriform bodies (fig. 17), which

¹Areschoug, Nova Acta., Reg. Soc. Ser. III, vol. x. Upsaliæ 1875.

²DeBary and Strasburger Bot. Zeit., Bd. xxxv (1877), p. 714.

on breaking out of the mother cell are seen to possess on their anterior end an eye spot and two cilia, by the rotation of which they dart actively here and there. These are the so-called *microzoospores*. Finally two of them from different parents, but in appearance precisely the same, come together and coalesce, their nuclei in this case, as in the last, probably fusing, but here only two uniting; while in the preceding stage there were more than two. This process we term conjugation, being the coalescence of two cells externally quite similar.

5. The next stage in the development is the union of more or less dimorphic elements. Both among plants and animals naturalists are agreed that it is impossible to draw any marked line of distinction between this and the preceding stage or conjugation. Sachs says "this differentiation presents a most complete series of gradations between the conjugation of similar cells and the fertilization of oospores by antherozoids, any boundary line between these processes being unnatural and artificial."

Cutleria, a seaweed of the branch *Oophyta*, is an interesting example of this stage. Here the female zoospores are large and borne singly in specialized cells in the parent. These on escaping, swim about for a time as do the microzoospores of *Ulothrix*, after which they come to rest. The smaller antherozoids now approach and conjugation takes place. In *Cutleria*, then, we have a union of differentiated cells for the first time, but they are yet both motile.

6. As an illustration of the next stage where we find complete differentiation as marked as in man, we select the Moss plant. In the mosses, the male and female organs are commonly borne on different plants. The egg cell is located at the bottom of a flask-shaped organ, the archegonium (fig. 18, *a*). The antherozoids (fig. 18, *b*) are small headed and biciliated, approaching in appearance very near to the spermatozoa of higher animals. In fertilization the antherozoids swim to and down the neck of the archegonium, at the bottom of which they find the quiescent oosphere or egg cell with which they fuse. The sexual cells of the moss plant, it is thus seen, unite two and two, as in the last case, but the differentia-

tion has been carried further, the female having become wholly incapable of independent motion, and the antherozoids have been gradually decreasing in comparative size. Here we have reached as high a development of fecundation as is probably found in the vegetable kingdom. (The stages in this development may be made clear by an examination of fig. 20, which is a modification of an illustrative diagram designed by Geddes and Thomson.)

I trust I have now made clear to you how fecundation probably originated, or rather the course it likely pursued in its gradual differentiation. Cell division, as we have seen, originated in almost a mechanical breaking apart of a mass of protoplasm. Conjugation and fecundation we now see, probably originated in the almost mechanical adhesion of the swarm spores of the *Acrasieæ*, followed by the mechanical fusion of the swarm spores of *Myxomycetes*, and gradually increasing in complexity until there is complete fusion (conjugation), then a fusion of elements differing in character. *Which is fecundation.*

DIFFERENTIATION OF SEX.

We may now direct our inquiry to the point in this evolution where sex becomes differentiated. In the conjugating swarm spores of the *Slime Molds* there seems to be no point where we can detect indications of a difference in the uniting individuals. So far as known there is no differentiation into male and female.

In *Ulothrix* (fig. 17) we begin to get a differentiation. In the conjugating microzoospores or planogametes (so called because of their similar character), it has been observed that planogametes produced in the same organ or gametangium will not coalesce with each other, but coalesce with planogametes from other gametangia. Here then, where the microscope fails to reveal any difference in the conjugating cells we nevertheless know from this fact that there must be some difference.

Ectocarpus siliculosus, one of the brown seaweeds, from the observations of Berthold, illustrates a rather different feature, by which we determine that the planogametes are really male

and female, although from external appearances we cannot recognize the difference between them. When the zoospores or planogametes are discharged from the mother cell, they do not differ by any morphological character. The females do not attract the males, but they swim around in the water and pass each other unnoticed. After a time, however, sex becomes manifest, and notably in accordance with the anabolic character of the female. Certain ones of the planogametes become motionless, draw in their cilia and assume a rounded shape (fig. 19, *a-c*). The female character of such cells is shown by the attraction they exert on the active males which collect about them in great numbers (a hundred or more), clustering at one side in a half circle. The anterior filament of each male is directed toward the female cell and is kept continually moving back and forth over it, the object being, it is thought, to provoke in the female planogamete genital excitation (fig. 19, *d*). After continuing to stroke the female for a time, one of the male planogametes leaves the circle and approaches the female, with which it gradually fuses, and fertilization is complete (fig. 19, *e-h*).

In the pond scums (*Spirogyra*, etc.), the reproduction of which is probably familiar to all, the filaments appear exactly alike, but the female character of one is shown by the cells of that filament containing all the spores resulting from the conjugation.

In *Outleria*, mentioned above, the difference is manifested by the size of the conjugating cells, but as we noticed, both male and female are still motile.

In the common rock weed, (*Fucus*—fig. 21), the differentiation becomes marked by the external forms of the sexual cells. The female cells are large and motionless, while the male cells are becoming more intensely male by a comparative decrease in size and increase, if anything, in vigor. By the vigor of their motions they give the oosphere, around which they collect in great numbers, a rotary motion for a time until it is fertilized.

In the *mosses* (fig. 18) and *ferns*, discussed above, we reach a complete and highly developed state of sexuality, probably more complete than in the higher flowering plants.

We have now traced hastily the course of the differentiation into the sexes, but the question "what causes this differentiation?" remains.

Starting with an amœboid cell let us see what changes environment might bring about in this direction. We have already seen that nourishment evidently has considerable to do in the determination of sex. Now the physiological conditions in reference to nourishment to which a cell may be subjected are evidently three: preponderating anabolism, preponderating katabolism or a medium between these when katabolism and anabolism are equal. Suppose an amœboid cell is subject to a preponderance of anabolism over katabolism the result would naturally be, increase in size, accompanied by a growing regularity of outline, increase in reserve food material and decreased mobility. The result is surely plain, we would have differentiated an *ovum* or egg cell. On the other hand subject the amœboid cell to preponderant katabolism, and we would as reasonably expect a decrease in size and in reserve materials accompanied by increased activity and the development of organs to aid in more rapid motion through the surrounding medium. In short in this manner we reach intelligibly the differentiation of sperm and ovum, antherozoid and oosphere (fig. 60, and explanations).

THE TWO SEXUAL ACTS IN SEA WEEDS.

In certain of the Red Seaweeds we appear to have the curious and unparalleled occurrence of two sexual acts in the life cycle of the plant, and the manner in which it is lead up to by transitional forms is very suggestive. The female reproductive organs which are borne on the same plant as the antheridia or on different ones consists usually of a group of cells, the *procarpium*, from one of which the egg cell proper, a long continuous closed tube, the trichogyne, grows out. In fertilization the spermatia are wafted about in the water until they come in contact with the trichogyne to which they adhere. The walls at the point of contact are absorbed, allowing the nucleus of the spermatia to pass over into the trichogyne and thus down to the egg cell, where it unites with the female pronu-

cleus (figs. 22 and 23). Shortly after fertilization a partition forms between the trichogyne and the egg cell, debarring the entrance of further spermatia and affording thus an excellent illustration of what Whitman has termed "Self-regulating receptivity." After fertilization the egg cell does not separate from its previous tissue connections, as in the oogonia of other green algæ, and the archegonia of the archegoniata, but remains in continuous connection with the hypogynal cells through which it is nourished.

In the simplest case (*Helminthocladiæ*) the ovacell develops from its surface many several-celled filaments, *ooblastemas*, as they are called, which form usually a closely compressed tuft. A single carpospore is developed at the apex of each of these ooblastema filaments (*Nemalion*, etc.) In this case, it will be noticed, all the ooblastema filaments are nourished through the egg cell.

In the *Gelidæ*, a slightly higher form, the fertilized ova from its surface cell develops a single filament, termed the ooblastema, which turns toward the axis of the branch and, ramifying abundantly, winds around this, sending branches into the highly nutritive outer layer of cells of the branch and connecting with some of these cells by the development of pits. Being thus abundantly nourished through this tissue, the branches of the ooblastema filament develop from each of the clavate erect terminal branch cells, either a single spore or short chains of two or more. In this case it is seen the ooblastema filament becomes in a sense parasitic upon the tissue of the parent plant.

In the families *Crytonemiæ* and *Squamariæ* a single or at least few ooblastema filaments develop from the fertilized egg cell. These creep about until they come in contact with certain specialized cells of the branch known as auxiliary cells, with which they enter into connection directly or by the development of conjugation processes. In many cases the union thus formed is limited to a fusion of the protoplasm while the cell nuclei remain separate, (*Dudresnaya*). In this case a process issues laterally from that half of the conjugation

cell which represents the ooblastema cell which by its further growth gives rise to the spore complex (fig. 22).

In other cases (*Gloeosiphonia*) when the contents of the ooblastema filaments flow into the auxiliary cell, the *nuclei unite*, the fusion or conjugation being thus complete. In this case the auxiliary cell separates off as an individual cell and gives rise to a lateral cell which becomes the centre of a spore complex (fig. 23).

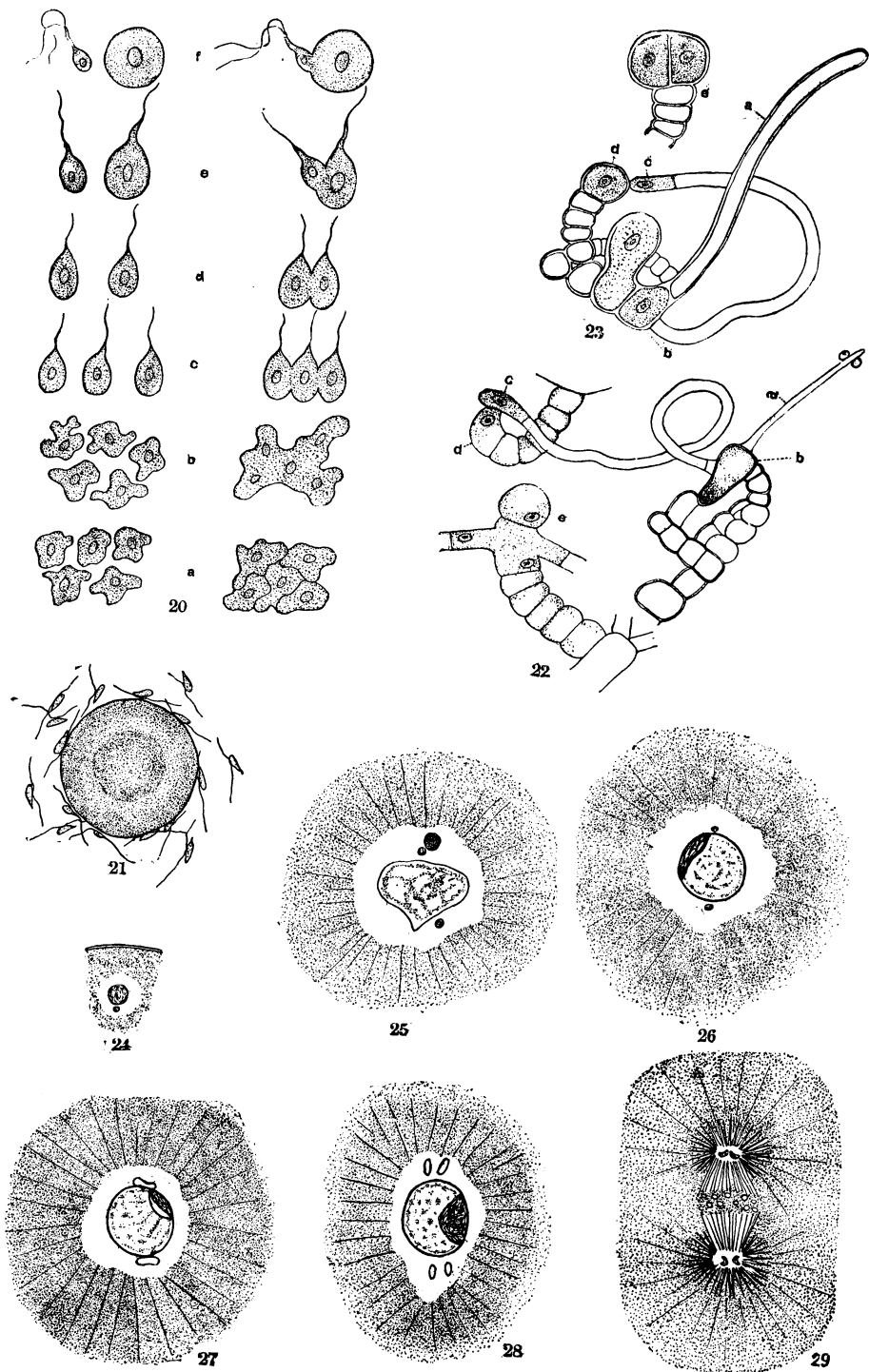
In the above case where the nuclei unite and where the conjugation gives an impulse to further development in the auxiliary cell, which otherwise would have remained quiescent, we have a case fulfilling all the requirements of a true sexual act,—true fecundation, and there seems to be no other way to consider this, than that here we have in the life cycle of the plant, two entirely different sexual acts, one following the other. We are surprised at this unprecedented phenomenon but we can not predicate why it should not occur. The reason for it we may assign to natural selection and development along natural lines. (1) The spores develop at the ends of filaments grown out from the egg cell. (2) The filaments thus formed begin to attach themselves to cells of the branch for nourishment. (3) We find special cells developed which the ooblastema filament finds and unites with in one sense but giving no nuclear union. (4) The ooblastema strikes a specialized cell with which it unites nuclei and protoplasm, the conjugation being complete and the further development from this auxiliary cell. May we not here in the development of the second sexual act of the Red Seaweeds derive a hint as to the physiological meaning of fecundation.

We start in a union for nutrition. We end with conjugation.

FECUNDATION IN ANIMALS.

Character of Ovum.—The animal egg or ovum presents all the characteristics of a normal somatic cell. The rather large nucleus is situated approximately in the centre of the cell, surrounded by abundant protoplasm. The abundant chromatin of the nucleus is arranged as in other cells in the form of a tangled coil like a disordered ball of twine. It is thought by

PLATE XII.



Fecundation and Development.

some, Van Beneden and others, to be continuous, but by Boveri and his followers it is maintained to be interrupted. This matters little, however, as the ultimate division of the coil is into a definite and regular number.

When the egg cell has attained its mature size, a peculiar occurrence takes place. The nucleus approaches the wall, forms a spindle and divides, forming at one side of the large ovum a tiny cell, containing half the nuclear matter and a small quantity of protoplasm from the ovum. This is not all, later a second spindle is formed and again the nucleus of the ovum divides throwing off another small cell. These cells thus given off from the ovum are known as *polar globules*. These little bodies, long passed by as of no importance, have by the masterly studies of later authors, foremost among whom are Van Beneden, Boveri and Weismann, been raised to a most important position and are intimately connected with late theories of fecundation.

Minot's Theory of Polar Globules.—What we may term Minot's theory assumes that in the cells both sexes are potentially present. To produce sexual elements the cell divides into its parts; in the case of the egg cell the male polar globules are cast off leaving the female ovum. In parthenogenetic ova he supposes that enough male matter is retained since only one polar globule appears to be formed. Van Beneden is also inclined to regard polar globules as eliminated male matter. Minot's theory then is that in every cell of every organism having sexual reproduction that there is an equal amount of female and of male matter, an equal number of male and of female chromatin bands; and that before the egg can be fertilized, it throws off the male matter that it contains as polar globules, so that the pronucleus consists merely of the female matter, of half the ordinary number of chromatin bands. The spermatozoon which has eliminated all female matter, enters and supplies the required amount of male matter. So that in the fecundated nucleus thus formed we have again the normal number of male and female chromatin segments from the different parents, and this nucleus by its segmentation forms every cell of the new organism. So

from this theory we arrive at an intelligible reason why the offspring comes to resemble both parents but there are difficulties in the way of further tracing heredity which we have not time here to consider.

Weismann's Theory.—Weismann's view is wholly different. He distinguishes in the ovum two kinds of plasm, the germ plasm and the histogenetic or ovogenetic nucleoplasm. The germ plasm which is at first present in the young egg he concludes originates first of all a special histogenetic or ovogenetic nucleoplasm which controls the egg cell up to the point of maturity, enabling it to secrete food material, develop membranes, etc. At maturity this ovogenetic nucleoplasm is of no more use and incapable of retransformation into germ plasm, and is hence thrown off by nuclear division forming the first polar globule. This is all that is extruded in the parthenogenetic ova. The second kind,—his germ plasm,—present in the egg, is that which enables the ovum to develop into an embryo. The second extrusion of a polar globule is a reduction of this germ plasm of the nucleus by half and the same must occur in the male germ cell also. What is thus lost in the formation of the second polar globule, is supplied by the fertilizing spermatozoön. The beginning of development depends, according to this hypothesis, upon the presence of a definite quantity of germ plasm. This the normal egg attains by first losing half and then regaining it; while the parthenogenetic egg attains the same result by never losing any. According to Weismann's view we see that only the second polar globule has to do directly with reproduction and here we have to look for an explanation of reproduction and heredity. As mentioned above Weismann looks upon the second polar globule, by which the germ plasm is reduced one half, as a reduction not only in quantity but above all in complexity of constitution, for by this means, he reasons, the excessive accumulations of different kinds of hereditary tendencies or germ plasms is prevented, which without it would necessarily be produced by fertilization. With the nucleus of the second polar body as many different kinds of germ plasms are removed from the egg as will be afterward added through the sperm nucleus. This

will likely need illustration to make it plain. Suppose we imagine an organism in which sex has just arisen and we thus have fertilization for the first time. In the egg cell resulting from this fertilization we would have mingled the germ plasms of but two parents, or but two kinds of chromatin in the nucleus; the chromatin, be it remembered, being the organ to which all such phenomena are traced. This daughter organism now conjugates with another similar individual which is also but one generation removed from the sexual origin. In the organism resulting from this union we obviously have commingled in the chromatin elements four ancestral tendencies or idioplasms. It is unnecessary to carry this further, obviously the next generation form a similar union, would contain 8 ancestral idioplasms, the next 16, the 10th generation 1024, and so on, doubling each time with every sexually produced generation. It is merely following the well known calculation made by breeders who merely differ in that they use the term blood, half blood or quarter blood, instead of germ, plasm or idioplasms as we have.

While in each succeeding generation the number of germ plasms are doubled, their quantities are reduced by one-half. Thus in a series of generations the continually recurring divisions of the ancestral germ plasms must theoretically ultimately reach a limit. So Weismann argues that the reduction in the number of chromatin bands accomplished by the formation of the second polar globule is to reduce by one-half the number of the ancestral germ plasms in the ovum, and the ancestral germ plasm added by the spermatozoan brings the number of germ plasms in the ovum up to the normal number which he supposes to be present. This theory is of course based on the almost universally accepted theory that fertilization consists in that an equal number of chromatin loops from either parent are placed side by side and form the new segmentation nucleus.

Character of Sperm.—The character of the spermatozoön is familiar to all. It consists of a minute head, composed chiefly of chromatin nuclear matter with a minimum allowance of cytoplasm and a long contractile tail which working behind

like a screw propeller, moves the essential head through the water or along the ducts.

Fertilization consists in a union of the spermatozoon with the ovum. Many devices are developed to bring the two cells near together, but they are then left to conjugate at will, as it were. The road that it is necessary for the spermatozoon to pass over to reach the ovum is frequently quite long, being in the hen about 60 cm. and in large mammals from 25-30 cm. But they are katabolic little creatures. It is wonderful how such frail creatures can manage to overcome such obstacles. Henle has seen spermatozoa carry along masses of crystals 10 times larger than themselves. Pouchet has seen them carry bunches of from eight to ten blood corpuscles. They have been estimated to carry burdens four or five times heavier than themselves without much difficulty or inconvenience.

*Foll's Observations on the Union of Pronuclei.*¹—Herman Foll describes the phenomena of fecundation in the egg of the sea urchin in about the following manner. The spermatozoon five minutes after entering the egg is conical and from its tip a small corpuscle, the spermocenter is detached (fig. 24). The spermatie pronucleus swells and approaches the female pronucleus the spermocentre in advance (fig. 25). The ovocenter is located on the side of the female pronucleus opposite to the side which gave rise to the polar globules. The spermocenter becomes placed at the pole on the side opposite the ovocenter (fig. 26). There are now two prolonged phases the "solar" and the "aureolar;" at the end of the first of these the ovocenter and spermocenter becomes divided in the form of "halters," as the author expresses it, which are not placed in the same plane. These "halters" come to lie parallel to each other in the plane which will be that of the aureole (fig. 27.) In the next phase the spermocenter and ovocenter become divided (fig. 28) and the halves passing in opposite directions along a fourth of a circumference of the combined nucleus arrive at a point at right angles to their previous position. This Foll calls the "Marche du quadrille."

¹Herman Foll. "Contribution a l'histoire de la fécondation," *Comptes Rendus Sci. T. cxii* (Avril 1891), p. 877.

At the moment when the demiovocenters and demispermo-centers are on the point of uniting, the aureole rapidly disappear and true aster become apparent with their perfectly distinct fibrils, much different from the radiations which are visible till then (fig. 29). The demicenters unite and fuse to form the first astrocenters.

The author concludes that fecundation consists not only in the addition of two nuclei arising from different individuals of different sexes, but in the union of two demispermo-centers with two demi-ovocentres to form the first two astrocenters. All succeeding astrocenters are derived in equal parts from the mother and father.

FECUNDATION IN HIGHER PLANTS.

Development of Embryo Sac and Egg Apparatus:—In the higher plants (the anthophytes or spermatophytes) we are particularly concerned with the embryo sac and its inclosed egg apparatus. It is necessary that we should thoroughly understand its development. The embryo sac first shows itself as an enlarged specialized cell in the upper central part of the nucellus or body of the ovule (fig. 30, a). In the maturation the nucleus divides and the two daughter nuclei thus formed travel in opposite directions, one going to the apex, the other to the base of the embryo sac which has, in the meantime, been growing larger and longer (fig. 31). After reaching their respective ends each divide again (figs. 32 and 33) and the two in each end thus formed again divide (fig. 34) forming a tetrad of nuclei at both the apex and the base of the embryo sac. Now a very peculiar thing happens. One of the nuclei from each tetrad thus formed leaves its position and journeys toward the centre of the embryo sac where they come together and fuse, forming the nucleus proper of the embryo sac (fig. 35, c). There is now left at each end of the embryo sac three nuclei of the original tetrad. The nuclei of the upper end become partitioned off by walls and form the egg apparatus proper. The two upper cells, the so-called *synergidæ* or accessory cells (fig. 35, a) are of doubtful function, being merely of secondary value in fertilization. They are some-

times capable, it has been observed, of being fertilized as egg cells and developing embryos (in cases of polyembryony). The lower cell (fig. 35, b) is the egg cell proper. The three basal cells become partitioned off by walls also and are known as antipodal cells; they appear to have no function in fertilization (fig. 35, d).

Development of Pollen:—The pollen or male germ cells are produced in great quantities in the pollen sacs of the anthers. They are formed in mother cells by two successive divisions of the nucleus, thus there are four pollen grains produced in each pollen mother cell (figs. 49 to 55). Later the nucleus of the pollen grains thus formed divides again (fig. 39) forming two cells in the grain, a small and a large one, the so-called generative and vegetative cells. The generative nucleus (fig. 40, b) of the small cell is the one important in fecundation. The vegetative nucleus (fig. 40, a) remains in the pollen grain having no further role in fecundation, or according to Guignard, sometimes passes into the pollen tube in advance of the generative nucleus and follows down the tube as it lengthens, until the micropyle is reached, when it gradually disorganizes and before fertilization takes place has disappeared. At first these two nuclei are separated by a cell wall but sooner or later the wall is broken down allowing the two nuclei to float free in the protoplasm of the pollen grain.

Reduction of the Number of Chromatin Elements in Sexual Nuclei.—Guignard¹ in a late article has emphasized the fact that in sexual cells there is a reduction in the number of the chromatin segments. In somatic cells he finds usually 24 segments, in the sexual cells the number is reduced to 12. In the formation of the young tissue of the anther 24 bands are uniformly present as far as the mother cells, the nucleus of which receives, as have the others so far 24 segments. After the complete differentiation of the mother cell it relapses for a time into a state of repose before the two divisions which are to form the pollen grain. When now the nucleus of the mother cell begins to manifest division it shows all the normal

¹Guignard, "Sur la Constitution des Noyaux Sexuels chez les Végétaux," *Comptes Rendus, Soc. de Biol.* 22. Mai 1891.—*Annal des Sci. Nat. Bot.* l. c.

changes of karyokenetic division but when the chromatin segments become visible there are only 12. The 12 segments are found again in the division which succeeds in order to form the pollen grain. Guignard assumes that during the formation of the mother cell the segments have united two and two either end to end or parallel; thus giving only 12. He thinks it certain that this reduction in number can in no way be connected with the elimination of nuclear matter as seen in polar globules.

In the nucellus of the ovule the nuclei all possess alike 24 chromatin segments. The cell which differentiates to form the embryo sac contains a nucleus which receives 24 chromatin segments but when the nucleus of this divides, after a long state of repose, they show a reduction, as in the pollen, from 24 to 12 segments and these 12 segments are found in all the succeeding divisions in the formation of the egg apparatus. A similar phenomenal reduction is said by Hertwig to occur in the animal kingdom, in the course of development of *Ascaris megaloccephala*.

Germination of Pollen and Growth of Pollen Tube.:—The act of fecundation proper consists in the union of the male nucleus of the pollen grain with the female nucleus of the egg cell. The mature pollen is transported from the anther where it is formed, to the stigma by the aid of insects, wind, water, etc. We are all, thanks to such men as Darwin and Lobbock, more or less familiar with the various processes by which pollination is accomplished. The pollen brought in contact with the stigma adheres there, being held and excited to germinate by a sticky, sugary exudation which covers the stigmatic surface.

In germination the pollen grain sends out a tube which grows down through the tissue of the style till it reaches the micropyle or entrance to the ovule through which it passes, enters the nucellus or body of the ovule, and comes in contact with the embryo sac, at the upper point where the egg apparatus is situated (figs. 36 and 37). It may well be asked how the pollen tubes in their blind chase downward succeed in finding such a small place as the micropyle and a single

cell in the body of the ovule. How in the inverted ovule they grow downward and then turn and retrace their steps upward; and the same question might properly be asked in regard to the lower plants. How does the antherozoöid of *Fucus* succeed in finding the oosphere which is floating, perhaps, at some distance in the water, or the antherozoöid of a moss plant, the small mouth of the archegonium which is on a different plant. As to this we can only suggest. It has been thought that in such cases the ovum secretes some substance which acts as a chemical excitant on the antherozoöids. Pfeffer working from this suggestion, has surely observed some interesting phenomena, that much strengthen if not absolutely confirm this hypothesis. His method of experimenting was this:—A solution of the substance to be experimented upon was placed in capillary tubes of from 5–7 hundredths of a millimeter wide. These capillary tubes dip into a watch crystal containing liquid wherein quantities of the antherozoids have been placed. Currents of diffusion will, it is seen, start up between the liquid in the capillary tube and that in the watch crystal, and when the substance experimented upon is the right one the antherozoöids are seen to follow the currents of diffusion and enter the capillary tube. In ferns Pfeffer found malic acid or malate to be the effective substance attracting the antherozoöids. As a proof of this, malic acid is found in abundance in prothallium decoctions of ferns and is known to be of very common occurrence throughout the vegetable kingdom. In the moss plant, cane sugar was found to be the effective substance and substances of the closest analogy as glucose, levulose, glycogen, etc., were found to exercise no attraction. Thus Pfeffer formulated an antherozoöid test for these substances, analogous to the bacteria test for oxygen invented by Englemann.

In the growth of the pollen tube from the stigma downward to the embryo sac, a conducting tissue is formed which accomplishes the same purpose. The conducting tissue consists of layers of specialized cells which become filled with nutritive saccharine material and furnishes nourishment to the pollen tube in its downward growth. Frequently we find continuous

tubes, lined by these specialized nourishing cells, leading from the stigma down to the cavity containing the ovules (fig. 38). In this case the ovary contains usually many ovules, frequently an enormous number, many hundreds indeed, so that it is necessary for many tubes to penetrate the style, as it requires one pollen tube to fertilize an ovule and there can be but little doubt but that almost every ovule formed in the ovary, receives a pollen tube and is fertilized. In the orchids one, by careful dissection, can find a silvery bundle of the pollen tubes and trace their progress from stigma to ovule.

In *Yucca* one can easily trace the long continuous tube that leads from the stigma down through the style, in the lower portion of which it branches into three parts, sending one branch into each cavity of the ovary. The pollen tubes may, with but little difficulty, be traced down through the stylar tube to the ovary cavity and found in numerous cases entering the micropyle of the ovules (figs. 36 and 37).

In some cases the irritation produced by the growing pollen tube through the tissue of the style, produces profound changes even before it reaches the egg cell and empties its contents. It has been observed, for instance in orchids, that at the time of pollination the ovules are in a very rudimentary condition and await the stimulus of the growing pollen tube, to develop the egg apparatus and prepare for fertilization. A month after the pollen tube starts its growth, the egg apparatus is completed and not until five weeks after this is fertilization completed. Similar phenomena have been observed in mullein, etc. It must not be thought that fecundation always requires so much time for its consummation, on the contrary it is usually a very quick process, requiring only a few days at most in flowering plants and much less in lower plants where the contact is direct.

Strasburger's Observations on the Immediate Process of Fecundation.—On reaching the embryo sac, the pollen tube hardly proceeds as we would expect. It does not penetrate into the egg cell and then burst leaving a free passage for the generative nucleus. In most cases, at least, it does not even penetrate the embryo sac but the end of the tube spreads out over the

apex of the embryo sac, covering the synergidæ (fig. 37). In some of the lower plants as *Peronospora* where the antheridium develops a conjugating tube, a direct passage is said to be formed by the bursting of the end of the tube which penetrates through the wall of the egg cell (fig. 59). The further process in flowering plants is, according to Strasburger, as follows: The nucleus of the generative cell of the pollen grain passes into the pollen tube and just before fertilization may be seen in the apex of the pollen tube surrounded by protoplasm. Before fertilization takes place this generative nucleus divides into two nuclei (figs. 41 and 42) and one of these passes out through the mucilaginous apex of the pollen tube and travels between the disorganized synergidæ to the oosphere. The generative nucleus then enters the oosphere, leaving behind it the protoplasm which had served as a vehicle, and fuses with the female pronucleus. Thus fecundation is completed and is as we see by this outlined process, a fusion of nuclei which would support the view that in reproduction the nuclei are the all important organs. The above description does not consider the existence of attractive spheres in the vegetable cell and as stated in our consideration of cell division, *Guignard*, followed by others, has lately asserted their universal occurrence accompanying the cell nucleus.

*Guignard's Discoveries.*¹—After its introduction into the pollen tube the generative nucleus is fusiform and surrounded by a layer of differentiated protoplasm. The directive spheres, two in number, are generally found at one end of the nucleus. When the generative nucleus divides into two, as explained above, after it has passed into the pollen tube and is located near the apex, the longer axes of the nuclear spindle, is always parallel to that of the pollen tube, hence that one of the resulting reproductive nuclei which is nearest the end of the tube has its attractive spheres preceding it. While the other on the contrary presents them behind the nucleus, where the other pole was situated. *Thus at the moment when the first of these cells, which alone is charged with fecundation, penetrates into*

¹Guignard, "Sur la Nature Morphologique du Phénomène de la Fécondation." *Compt. Rend. de Biol.* 9 Ser. T. 111 (1891) p. 467.—*Annal. des-Sci. Nat. Bot.* 1. c.

the female apparatus the two directive spheres which it possesses precede it.

In the embryo sac, as explained above, one stage shows the nuclei disposed in two tetrads, one at the summit and the other at the base. In the apical tetrad the nuclei which belong to the synergidæ are formed by a horizontal division (figs. 43 and 44) and their attractive spheres, therefore, occupy their lateral faces. The two other nuclei on the contrary are originated in a perpendicular plane (fig. 43). Thus the nucleus which goes to form the oosphere, has its two attractive spheres above it (figs. 44 and 45, *b*) while the other that travels to the centre of the embryo sac to fuse with a similar one from below, has its attractive spheres below it. (Upper nucleus, fig. 44, *c*.)

The male nucleus, which is strongly contracted in its passage into the egg cell, increases in size and forms what is now termed the male pronucleus which is preceded, it will be remembered, by its two attractive spheres (fig. 45, *d*). The contact first occurs between the attractive spheres. These coalesce two by two, male sphere with female sphere (fig. 46). They then separate from each other so as to allow the male and female pronuclei to pass between them and fuse (fig. 47 and 48). The male pronucleus unites with the female pronucleus and remains thus in contact but is clearly distinguishable until the first segmentation starts. In each couple, formed by the union of male and female attractive spheres, the fusion takes place slowly. When thoroughly fused fecundation is complete. The two new spheres thus formed will be the origin of the poles of the first segmentation spindle. Before the first division they orient themselves in such a fashion that this spindle will be parallel to the longitudinal axes of the egg cell. *It results, from these observations, that the phenomena of fecundation consists not only in the copulation of two nuclei of different sexual origin but also in the fusion of two protoplasmic bodies of equally different origin.*

The process of fecundation in the sea urchin, explained above from Foll, agrees chiefly with this. Differing only in that the attractive sphere does not divide until it enters the egg.

Polar Globules in Plants.—Have we now anything in the

maturation of the egg cell and the antherozoids that correspond to polar globules in the animal egg? In short, it is thought that we find analogous exudations of nuclear matter almost universally in plants. In the development of the planogametes of *Ulothrix* a portion of waste protoplasm is extruded with the planogametes containing probably the extruded nuclear matter.

Among the well differentiated female gametes, it is said, *Peronospora* affords an excellent illustration of what we may term polar globules. In the development of the oosphere, according to Wagner, the numerous nuclei which at first are scattered uniformly throughout the oogonium (fig. 57) at length approach the periphery leaving the central portion of the oogonium occupied by large vacuoles, and a small central mass of protoplasm connected with the periphery by protoplasmic strands. The nuclei now limited to the periphery further divide and 2 or 3 (?) finally leave the periphery and approach the central mass of protoplasm traveling along the connecting protoplasmic strands and supposedly unite in the centre, forming the nucleus of the oosphere (fig. 58). While these nuclei are thus traveling toward the centre, the cell wall of the oosphere begins to form, separating the central mass of protoplasm with its two nuclei from the peripheral or *periplasm* with its numerous nuclei, some of which are supposed to be used up in the formation of the oosphere wall. The nuclei thus relegated to the periplasm have been considered as of the nature of polar globules. But with the present light on the subject it must remain surely as a very doubtful and indefinite case.

In the development of the antherozooids of ferns, when the antherozooid is set free there is attached to its posterior end an appendage which is usually described as a protoplasmic vesicle, but Dodel-Port and Belajeff think it to contain nuclear matter also from the mother cells.

In flowering plants the nucleus of the pollen divides into two cells (figs. 39 and 40) a vegetative and a generative. The vegetative is thought by Strasburger to have the function of a polar globule. Again the generative

nucleus after it has passed down the pollen tube divides, and only one portion enters the oosphere (figs. 41 and 42), the other remaining as waste nuclear matter and may be considered as a second polar globule. In the development of the embryo sac it is thought the division of the nucleus which gives rise to the nucleus of the egg cell and of the polar nucleus which travels to the middle of the embryo sac to fuse with a similar one from the basal tetrad, is one of the divisions sought and that the polar nucleus has in reality the significance of a polar globule. If a second polar globule is formed it is likely the preceding division, that which gives rise to the first of the synergidæ. If this is the case the regular after division that forms the two synergidæ may be looked upon as corresponding with the rather abnormal but quite frequent division which occurs in the first polar globule of many animals.

The instances cited of polar globules in the vegetable kingdom, it will be seen, do not possess that definiteness that is found in the animal kingdom. We fain would have greater definiteness but further work is here necessary. Doubt clusters about many questions connected with fertilization. We must be content to take things as they are even if somewhat unsatisfactory, until further investigation throws light on the obscure points.

Shaw School of Botany.

December 16, 1891.

St. Louis, Mo.

EXPLANATION OF PLATES.

The figures are mostly redrawn or adapted from various standard investigations. A few are original.

PLATES XI-XIV.

Fig. 1. A portion of the frond of *Caulerpa*, natural size. (Redrawn from Sachs' *Phys. of Plants*, Eng. Ed. p. 492.

Figs. 2-9. *Iris pumila*. Dividing mother cell of a stoma. (Redrawn from Strasburger "*Zellbild. und Zellteil.*" (3rd. Ed. 1880.) Pl. viii). *Fig. 2.* Resting nucleus. *3.* Contraction and breaking up of the nuclear thread. *4.* Nuclear spindle. *5.*

Separation of chromatin segments. 6. Fusion of chromatin segments at each pole.. 7 and 8. Organization of the daughter nuclei and formation of the cell plate. 9. The two resulting daughter nuclei.

Figs. 10–11. (Redrawn from Watase.—“Karyokinesis” Biological Lectures p. 168). Fig. 10. Nucleus dividing, showing archoplasmic spheres; (a) centre of archoplasm; (c) cytoplasm. Fig. 11. Division of the archoplasmic sphere.

Fig. 12. (Original). Diagrammatic outline of nuclear division modeled from Guignard's description; (a) centrosome surrounded by a hyaline circle; (b) surrounding granular circle; (c) cytoplasm.

Fig. 13. (Original). Diagrammatic outline of the development of the *Acrasieæ*; (a) spore; (b) escaping mass of protoplasm; (c) swarm spore; (d) swarm spore preparing for division; (e) dividing swarm spore; (f) completion of the division; (g) plasmodium; (h) sporangium.

Fig. 14. (Original). Diagrammatic outline of the plasmodium formation, etc. in *Myxomycetes*; (a) swarm spores; (b) starting of fusion; (c) plasmodium; (d) sporangium.

Figs. 15–16. (Redrawn from Strasburger Bot. Zeit. xxxv, (1877) Taf. xiii, fig. 14, f. and i.) Multiple conjugation of the zoospores of *Acetabularia mediterranea*.

Fig. 17. (Redrawn from Dodel-Port in Vines. Phys. Bot. p. 606) Planogametes of *Ulothrix*, one free, others in conjugation.

Fig. 18. (Redrawn from Strasburger) Reproduction of a moss plant; (a) archegonium with enclosed egg cell; (b) antherozooid.

Fig. 19. (Redrawn after Berthold in Binet's Psychic Life of Micro-Organisms p. 84) Planogametes of *Ectocarpus siliculosus*. (a–c) Differentiation of female planogamete; (d) female planogamete surrounded by males; (e–h) process of conjugation.

Fig. 20. (Adapted from Geddes and Thomson in Evolution of Sex.) Diagram of the course of development of fecundation. (a) Adhesion of swarm spores into plasmodium, ex. *Acrasieæ*; (b) fusion of swarm spores into plasmodium, ex. *Myxomycetes*; (c) multiple conjugation of planogametes, ex. *Acetabularia*; (d) conjugation of two planogametes, ex. *Ulothrix*; (e) conjugation of dimorphic notile cells, ex. *Cutleria*; (f)

fecundation proper of egg cell by antherozooid, ex. *Moss Plant*.

Fig. 21. (Redrawn from Thuret in Bessey's Bot. p. 267). Oosphere of *Fucus vesiculosus* surrounded by spermatozooids.

Fig. 22. (Adapted from Schmitz Ann. and Mag. of Nat. Hist. vol. xiii, Ser. 5, (1884) Pl. 1, figs. 16-19) *Dudresnaya purpurifera*. (a) Trichogyne with adhering spermatia; (b) egg cell; (c) conjugating cell cut off at end of ooblastema filament; (d) auxiliary cell; (e) later stage after conjugation of auxiliary cell and ooblastema filament.

Fig. 23. (Adapted from Schmitz, l. c.) *Glaeosiphonia capillaris*, (letters as in fig. 22.)

Figs. 24-29. Redrawn from Foll, Comptes Rendus l. c.) Fecundation of the egg of the sea urchin.

Figs. 30-35. (Redrawn from Strasburger, Zellbild, und Zellteil, 3 Auflage, Pls. iv and v). Development of the embryo sac and egg apparatus of *Monotropa hypopitys*. *Fig. 30.* Nucellus with embryo sac; and (a) its primary embryo sac nucleus. *Fig. 31-34,* enlargement of embryo sac and formation of the two apical tetrads of nuclei. *Fig. 35.* (a) synergidæ; (b) egg cell; (c) the two nuclei, one from each tetrad that fuse forming the nucleus proper of the embryo sac; (d) antipodal cells.

Fig. 36. (Original). Camera sketch of a longitudinal section of the pistil of *Yucca angustifolia*, x. about 5 diam. Showing the continuous styler tube with numerous pollen tubes running down to the ovules.

Fig. 37. (Original). Camera sketch, x. 400 diam. of the ovule of *Yucca angustifolia*, showing one ovule coat, the nucellus, the embryo sac with its enclosed egg apparatus, and a pollen tube; (e) that has entered the micropyle of the ovule and penetrated to the embryo sac.

Fig. 38. (Original). Conducting tissue. Cross section of the style of *Yucca angustifolia*. Camera sketch, x. 150 diam.

Figs. 39-42. (Redrawn from Strasburger Befrucht. bei den Phaner. Taf. 1.) *Fig. 39.* Young pollen grain during division into generative and vegetative cells. *Fig. 40.* Mature pollen grain; (a) vegetative nucleus; (b) generative nucleus. *Figs. 41 and 42.* Portions of the pollen tube with the generative nucleus in division.

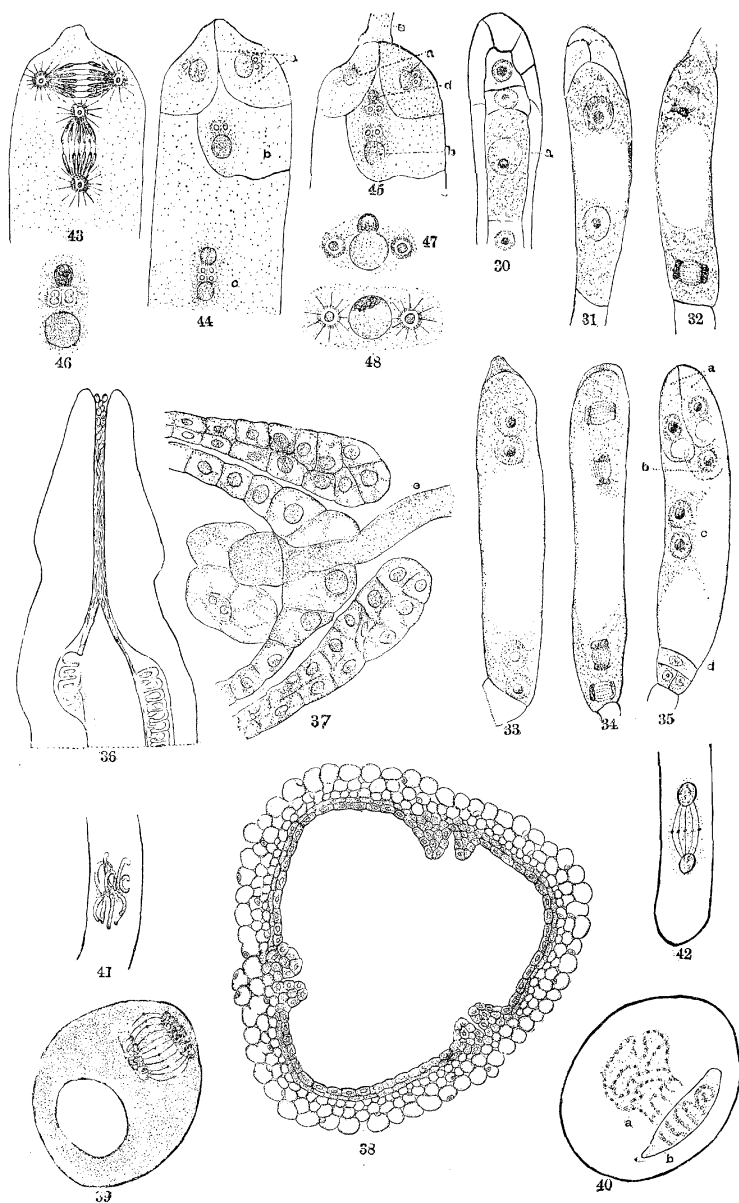
Figs. 43-48. (Original). Diagrammatic outlines of embryo sac and nuclear changes during fecundation modeled from Guignard's descriptions. Fig. 43. Division of nuclei to form the upper tetrad, showing asters centrosomes, etc. Fig. 44. (a) synergidæ; (b) oosphere; (c) union of nuclei to form the nucleus of the embryo sac. Fig. 45. (a) nuclei of the synergidæ; (b) nucleus of the oosphere; (d) male pronucleus preceded by its directive spheres; (e) pollen tube. Fig. 46. Union of the male and female directive spheres. Fig. 47. Separation of the directive spheres and union of nuclei. Fig. 48. Fecundated nucleus ready for the first segmentation, the male portion is still distinguishable (the male nucleus in the last three colored dark).

Figs. 49-56. (Redrawn from Guignard, *Annal. des Sci. Nat. Bot. Se. 7, T. xiv, Pl. 10*). Formation of the pollen grains in a pollen mother cell of *Lilium martagon*. Fig. 49. Mother cell in resting stage showing chromatin band, nucleolus (paranucleolus) and two directive spheres. Fig. 50. Rupture of chromatin filament into 12 segments. Fig. 51. Division (longitudinal) of these segments. Fig. 52. The nuclear spindle in profile. Fig. 53. Separation of the daughter segments and division of the directive sphere. Fig. 54. Two cells in the resting stage, completion of the first division. Fig. 55. Division of these two cells to form the four pollen grains. Fig. 56. One of the young pollen grains of the last division in a resting stage before the division which gives rise to the vegetative and generative nuclei. (See Fig. 39.)

Figs. 57-59. (Redrawn from Wagner, *Ann. of Bot. vol. iv, Pl. vi.*) Fecundation of *Peronospora parasitica*. Fig. 57. Oogonium with antheridium at one side. Fig. 58. Formation of oosphere, two nuclei approaching the centre to unite. Fig. 59. Mature oosphere in process of fecundation, showing the antheridial tube grown through the oogonium to the oosphere.

Fig. 60. (Adapted from Geddes and Thomson, *l. c.*) Diagram illustrating effect of environment on an amœboid cell. On the left, when subjected to preponderating katabolism, yielding *antherozooid*. On the right when subjected to preponderating anabolism, yielding oosphere. Medium conditions indicated by the central line of amœboid cells.

PLATE XIII.

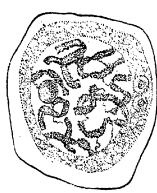


Fecundation and Development.

PLATE XIV.



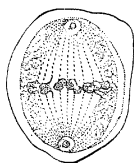
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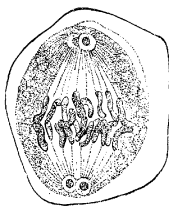
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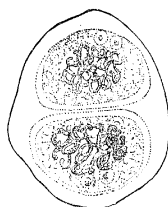
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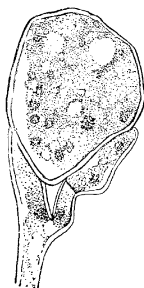
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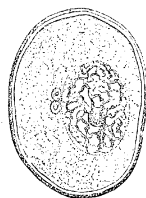
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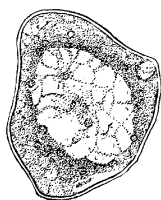
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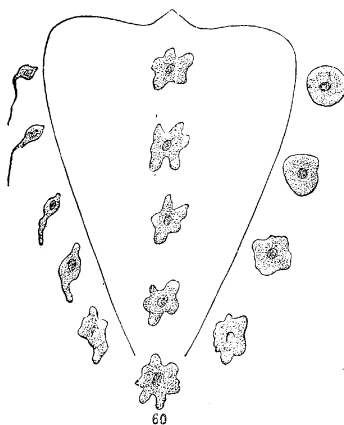
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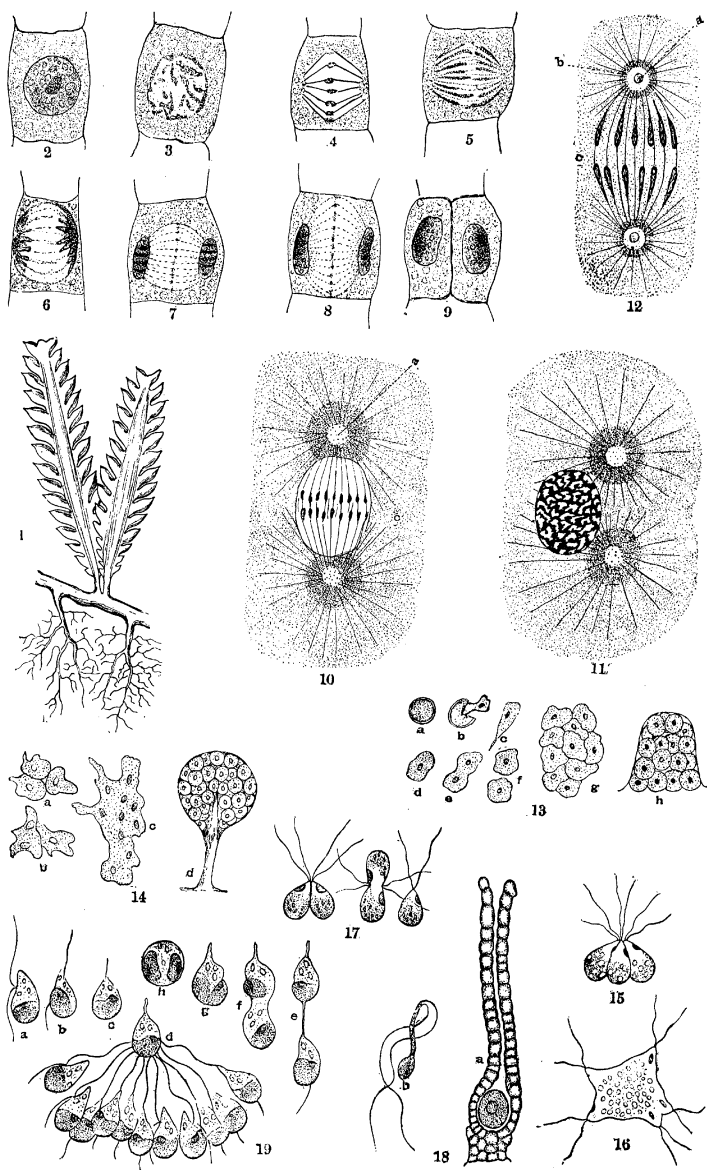
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PLATE XI.



Fecundation and Development.